

Nucleotide and Amino Acid Sequences of Rat HICP

| | |
|---|-----|
| GACGCTTCTG ATCTCCAGAG GACCCTGGGG TGGGACAGGG GCCTTGCCAA GGCTGCAGCC | 60 |
| GCTGGGCAGT GGCTTGGAAT GGAGGTCTTT ATTACTGGGA ACTGAGGAGC TAAGAGGCTC | 120 |
| CTGTCAGCTT GTCCTAAAGT CTTAGCACTT GTGGTGGCTT GGGCTTCACA CACTGTCAGA | 180 |
| CACCTTCGTG GTGGCCTCCA CGGCCTCACC TTCAGGTTTG AAGCTGGCTC CACAAGGGAC | 240 |
| ACGGTGAC ATG AGG GGC AGC CCA CTG ATC CAT CTT CTG GCC ACT TCC TTC | 290 |
| Met Arg Gly Ser Pro Leu Ile His Leu Leu Ala Thr Ser Phe | |
| 1 5 10 | |
| CTC TGC CTT CTC TCA ATG GTG TGT GCC CAG CTG TGC CGG ACA CCC TGT | 338 |
| Leu Cys Leu Leu Ser Met Val Cys Ala Gln Leu Cys Arg Thr Pro Cys | |
| 15 20 25 30 | |
| ACC TGT CCT TGG ACA CCA CCC CAG TGC CCA CAG GGG GTA CCC CTG GTG | 386 |
| Thr Cys Pro Trp Thr Pro Pro Gln Cys Pro Gln Gly Val Pro Leu Val | |
| 35 40 45 | |
| CTG GAT GGC TGT GGC TGC TGT AAA GTG TGT GCA CGG AGG CTG GGG GAG | 434 |
| Leu Asp Gly Cys Gly Cys Cys Lys Val Cys Ala Arg Arg Leu Gly Glu | |
| 50 55 60 | |
| TCC TGC GAC CAC CTG CAT GTC TGC GAC CCC AGC CAG GGC CTG GTT TGT | 482 |
| Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln Gly Leu Val Cys | |
| 65 70 75 | |
| CAG CCT GGG GCA GGC CCT GGC GGC CAT GGG GCT GTG TGT CTC TTG GAT | 530 |
| Gln Pro Gly Ala Gly Pro Gly Gly His Gly Ala Val Cys Leu Leu Asp | |
| 80 85 90 | |
| GAG GAT GAC GGT AGC TGT GAG GTG AAT GGC CGC AGG TAC CTG GAT GGA | 578 |
| Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly | |
| 95 100 105 110 | |
| GAG ACC TTT AAA CCC AAT TGC AGG GTC CTG TGC CGC TGT GAT GAC GGT | 626 |
| Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly | |
| 115 120 125 | |
| GGC TTC ACC TGC CTG CCG CTG TGC AGT GAG GAT GTG CGG CTG CCC AGC | 674 |
| Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser | |
| 130 135 140 | |
| TGG GAC TGC CCA CGC CCC AAG AGA ATA CAG GTG CCA GGA AAG TGC TGC | 722 |
| Trp Asp Cys Pro Arg Pro Lys Arg Ile Gln Val Pro Gly Lys Cys Cys | |
| 145 150 155 | |
| CCC GAG TGC GTA TGT GAC CAG GGA GTG ACA CCG GCG ATC CAG CGC TCC | 770 |
| Pro Glu Trp Val Cys Asp Gln Gly Val Thr Pro Ala Ile Gln Arg Ser | |
| 160 165 170 | |
| ACG GCG CAA GGA CAC CAA CTT TCT GCC CTT GTC ACT CCT GCC TCT GCT | 818 |
| Thr Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala | |
| 175 180 185 190 | |
| GAT GCT CCT TGT CCA AAT TGG AGC ACA GCC TGG GGC CCC TGC TCA ACC | 866 |

FIGURE 1

Nucleotide Sequence Encoding Mature HICP and the Amino Acid Sequence of Mature HICP

| | |
|---|-----|
| CAG CTG TGC CGG ACA CCC TGT ACC TGT CCT TGG ACA CCA CCC CAG TGC | 48 |
| Gln Leu Cys Arg Thr Pro Cys Thr Cys Pro Trp Thr Pro Pro Gln Cys | |
| 1 5 10 15 | |
| CCA CAG GGG GTA CCC CTG GTG CTG GAT GGC TGT GGC TGC TGT AAA GTG | 96 |
| Pro Gln Gly Val Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Lys Val | |
| 20 25 30 | |
| TGT GCA CGG AGG CTG GGG GAG TCC TGC GAC CAC CTG CAT GTC TGC GAC | 144 |
| Cys Ala Arg Arg Leu Gly Glu Ser Cys Asp His Leu His Val Cys Asp | |
| 35 40 45 | |
| CCC AGC CAG GGC CTG GTT TGT CAG CCT GGG GCA GGC CCT GGC GGC CAT | 192 |
| Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Gly Gly His | |
| 50 55 60 | |
| GGG GCT GTG TGT CTC TTG GAT GAG GAT GAC GGT AGC TGT GAG GTG AAT | 240 |
| Gly Ala Val Cys Leu Leu Asp Glu Asp Asp Gly Ser Cys Glu Val Asn | |
| 65 70 75 80 | |
| GGC CGC AGG TAC CTG GAT GGA GAG ACC TTT AAA CCC AAT TGC AGG GTC | 288 |
| Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val | |
| 85 90 95 | |
| CTG TGC CGC TGT GAT GAC GGT GGC TTC ACC TGC CTG CCG CTG TGC AGT | 336 |
| Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser | |
| 100 105 110 | |
| GAG GAT GTG CGG CTG CCC AGC TGG GAC TGC CCA CGC CCC AAG AGA ATA | 384 |
| Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Lys Arg Ile | |
| 115 120 125 | |
| CAG GTG CCA GGA AAG TGC TGC CCC GAG TGG GTA TGT GAC CAG GGA GTG | 432 |
| Gln Val Pro Gly Lys Cys Cys Pro Glu Trp Val Cys Asp Gln Gly Val | |
| 130 135 140 | |
| ACA CCG GCG ATC CAG CGC TCC ACG GCG CAA GGA CAC CAA CTT TCT GCC | 480 |
| Thr Pro Ala Ile Gln Arg Ser Thr Ala Gln Gly His Gln Leu Ser Ala | |
| 145 150 155 160 | |
| CTT GTC ACT CCT GCC TCT GCT GAT GCT CCT TGT CCA AAT TGG AGC ACA | 528 |
| Leu Val Thr Pro Ala Ser Ala Asp Ala Pro Cys Pro Asn Trp Ser Thr | |
| 165 170 175 | |
| GCC TGG GGC CCC TGC TCA ACC ACC TGT GGG CTG GGC ATA GCC ACC CGA | 576 |
| Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg | |
| 180 185 190 | |
| GTG TCC AAC CAG AAC CGA TTC TGC CAA CTG GAG ATC CAA CGC CGC CTG | 624 |
| Val Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu | |
| 195 200 205 | |
| TGT CTG CCC AGA CCC TGC CTG GCA GCC AGG AGC CAC AGC TCA TGG AAC | 672 |
| Cys Leu Pro Arg Pro Cys Leu Ala Ala Arg Ser His Ser Ser Trp Asn | |
| 210 215 220 | |

FIGURE 2

AGT GCT TTC
Ser Ala Phe
225

681

AGT GCT TTC
Ser Ala Phe
225

FIGURE 2 (Continued)

Alignment of the Modular Domains of HICP with the Modular Domains of Other CCN Family Members

MODULE I : IGFBP Domain

| | | | | | | | | | | |
|---------|---------------------|----------------|-----------------|------------------|------------|----|----|----|----|-----|
| | 28 | 45 | 46 | 60 | 61 | 75 | 76 | 90 | 91 | 100 |
| 1 HICP | QICRTPCT--CP-WTPPQC | -PQVPLVLDGGCC | KVCARRLGESCDHLH | VCDFPSQGLVCQPGAG | PGGHGAVCLL | | | | | |
| 2 CTGF | QDCSAQCQ--CAAEAAPHC | -PAGVSLVLDGGCC | RVCAKQLGELCTERD | PCDPHKGLFCDFGSP | ANRKIGVCTA | | | | | |
| 3 NOV | LRCPSRCPPKCPIS-PTC | AP-GVRSVLDGSCC | PVCARQGESCSSEMR | PCDQSSGLYCDRSAD | PNNQTGICMV | | | | | |
| 4 CYR61 | -TCPAACH--CPLEA-PKC | AP-GVGLVRDGGCC | KVCAKQLNEDCSKTQ | PCDHTKGLECNFGAS | STALKGICRA | | | | | |

MODULE II : vWFC Domain

| | | | | | | | | | | |
|---------|----------------------|-----------------|-----------------|-----------------|-----------------|-----|-----|-----|-----|-----|
| | 101 | 120 | 121 | 135 | 136 | 150 | 151 | 165 | 166 | 180 |
| 1 HICP | DGSCCEVNGRRYLDGETFKP | NCRVLCRCDDGGFTC | LPLCSEDVRLPSWDC | PRFKRIQVPGKCCPE | WVC | | | | | D-Q |
| 2 CTGF | DGAPCVFGGSVYRSGESFQS | SKYQCTCLDGAVGC | VPLCSMDVRLPSDC | PFPRVKLPKCKCKE | WVC | | | | | DEP |
| 3 NOV | EGDNCVFDGVIYRNGEKFEF | NCQYFCTCRDQIGC | LPRQLDVLLPGPDC | PAPRKVAVPGECCEK | WTCGS | | | | | DEQ |
| 4 CYR61 | EGRPCEYNSRIYQNGESFQP | NCKHQCTCIDGAVGC | IPLCQELSLPNLGC | PNRLVKVSGQCCEE | WVCDEDSIKDSLDDQ | | | | | |

MODULE III : TSP1 Domain

| | | | | | |
|---------|------------|-----------------|-----------------|----------------------|-----|
| | 240 | 250 | 265 | 280 | 298 |
| 1 HICP | PCPNWSTAWG | PCSTTCGLGIATRVS | NQNRFCQLEIQRRLC | LPRPCLAAARSHSSWNSAF- | |
| 2 CTGF | NCLVQTTEWS | ACSKTCGMGISTRVT | NDNTFCRLEKQSRLC | MVRPCEADLEENIK-KGKK | |
| 3 NOV | NCIEQTTEWS | ACSKSCGMGVSTRVT | NRNRQCEMVKQTRLC | IVRPCEQEPEEVDKKGKK | |
| 4 CYR61 | KCIVQTTSWS | QCSKSCGTGISTRVT | NDNPECRLVKETRIC | EVRPCGQPVYSSLK-KGKK | |

FIGURE 3

Northern Blot Analysis of HICP Expression in Rat Aorta Smooth Muscle Cells

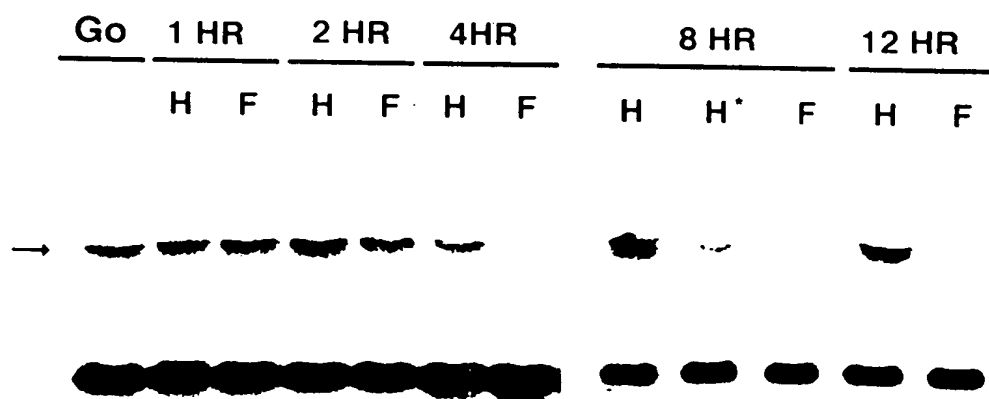


FIGURE 4

DNA Synthesis in Rat Aorta Smooth Muscle Cells

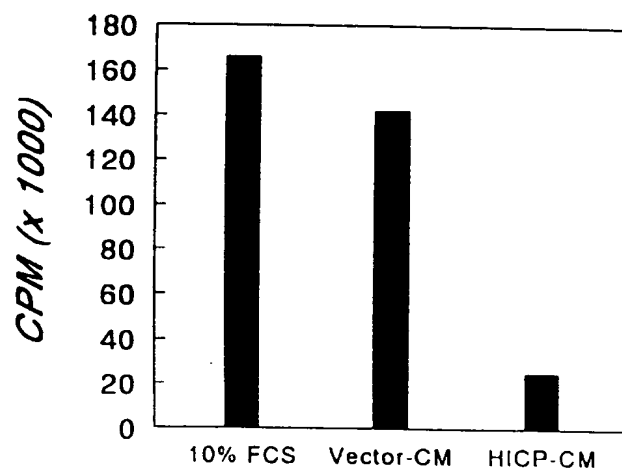


FIGURE 5